

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Goff *et al.*

Serial. No. TBD

Filed: Concurrently Herewith

For: Control Of Gene Expression In Plants By  
Receptor Mediated Transactivation In The  
Presence Of A Chemical Ligand

Art Unit: TBD

Examiner: TBD

Atty Docket: S-20359D

**PRELIMINARY AMENDMENT**

Commissioner of Patents  
Washington, D.C. 20231

Sir:

**IN THE SPECIFICATION**

Please insert the following specification paragraphs in re-written "clean form" as follows:

**Paragraph 1 on page 1 of the specification. This is a newly inserted paragraph immediately following the title.**

-- This is a continuation of Application No. 09/625,904, filed July 26, 2000, which is a continuation of Application No. 09/234,190, filed January 20, 1999, now Patent No. 6,147,282, which is a division of Application No. 09/010,050, filed January 21, 1998, now Patent No. 5,880,333, which is a continuation of Application No. 08/398,037, filed March 3, 1995, abandoned. --.

**Paragraph 1 on page 10 of the specification.**

--binding domain. The steroid hormone ecdysone triggers coordinate changes in tissue development that results in metamorphosis, and ecdysone has been shown to bind to EcR. Koelle *et al.* *Cell* 67: 59-77, 1991. The plant-produced analog of ecdysone, muristerone, also binds to the ligand binding domain of EcR. Other chemicals, such as the non-steroidal ecdysone agonists RH 5849 (Wing, *Science* 241: 467-469 (1988)) and RH 5992 (tebufenozide), the latter known as the insecticide MIMIC<sup>®</sup>, also will act as a chemical ligand for the ligand binding domain of EcR. The EcR and its ligand binding domain have been found in the present invention to be particularly useful for controlling target polypeptide expression in plant cells, as described in the examples below.--

**Paragraph 2 on page 16 of the specification.**

--Tissue-specific or tissue-preferential promoters useful in the present invention in plants, particularly maize, are those which direct expression in root, pith, leaf or pollen. Such promoters are disclosed in Patent No. 5,625,136, herein incorporated by reference in its entirety. Also useful are promoters which confer seed-specific expression, such as those disclosed by Schernthaner *et al.*, *EMBO J.* 7: 1249 (1988); anther-specific promoters ant32 and ant43D disclosed in Patent No. 5,477,002, herein incorporated by reference in its entirety; anther (tapetal) specific promoter B6 (Huffman *et al.*, *J. Cell. Biochem.* 17B: Abstract #D209 (1993)); pistil-specific promoters such as a modified S13 promoter (Dzelkalns *et al.*, *Plant Cell* 5:855 (1993)).--

**Paragraph 3 on page 16 of the specification.**

--Also useful in the present invention are chemically-induced promoters. Particular promoters in this category useful for directing the expression of the receptor polypeptides or target polypeptide in plants are disclosed, for example, in Patent No. 5,614,395, herein incorporated by reference in its entirety.—

**Paragraph 1 on page 19 of the specification.**

--Serial No. 08/008,374, herein incorporated by reference in its entirety. An additional preferred embodiment is the protoplast transformation method for maize as disclosed in European Patent Application EP 0 292 435, as well as in Patent No. 5,350,689, hereby incorporated by reference in its entirety. One particularly preferred set of embodiments for the introduction of the expression cassettes of the present invention into wheat by microprojectile bombardment can be found in U.S. Serial No. 08/147,161, herein incorporated by reference in its entirety.--

**IN THE CLAIMS**

Please cancel claims 1-25, 27, and 36 without prejudice or disclaimer.

Please amend claims 26, 28, 31, and 32 as follows in re-written "clean" form:

26. (Amended) A receptor expression cassette for use in a method of controlling gene expression in a plant, comprising:
- a) a 5' regulatory region capable of promoting expression in a plant cell;
  - b) an operably linked coding sequence encoding a receptor polypeptide comprising a ligand binding domain and a DNA binding domain, wherein said receptor polypeptide is a member of the Class II steroid and thyroid hormone superfamily of nuclear receptors; and
  - c) a 3' terminating sequence.
28. (Amended) The receptor expression cassette of claim 26 wherein said receptor polypeptide is Ecdysone Receptor.
29. The receptor expression cassette of claim 28 wherein said receptor polypeptide further comprises a heterologous transactivation domain.

30. The receptor expression cassette of claim 29 wherein said heterologous transactivation domain is the transactivation domain from the C1 regulatory gene of maize.
31. (Amended) The receptor expression cassette of claim 28 wherein said DNA binding domain is heterologous with respect to said receptor polypeptide.
32. (Amended) The receptor expression cassette of claim 31 wherein said heterologous DNA binding domain is the DNA binding domain from the GAL4 protein of yeast.
33. The receptor expression cassette of claim 26 wherein said receptor polypeptide is USP.
34. The receptor expression cassette of claim 33 wherein said receptor polypeptide further comprises a heterologous transactivation domain.
35. The receptor expression cassette of claim 34 wherein said heterologous transactivation domain is the transactivation domain from the VP16 protein of herpes simplex.

**Please add new claims 37-41 as follows in re-written "clean form":**

37. The receptor expression cassette of claim 26 wherein said receptor polypeptide further comprises a heterologous transactivation domain.
38. The receptor expression cassette of claim 26 wherein said DNA binding domain is heterologous with respect to said receptor polypeptide.
39. A plant transformation vector comprising the receptor expression cassette of claim 26.
40. A plant transformed with the plant transformation vector of claim 39.

41. Seed from the plant of claim 40.

**Version with markings to show changes made.**

**A marked up version of the paragraph added to page 1 of the specification follows:**

--This is a continuation of Application No. 09/625,904, filed July 26, 2000, which is a continuation of Application No. 09/234,190, filed January 20, 1999, now Patent No. 6,147,282, which is a division of Application No. 09/010,050, filed January 21, 1998, now Patent No. 5,880,333, which is a continuation of Application No. 08/398,037, filed March 3, 1995, abandoned.—

**A marked up version of paragraph 1 on page 10 of the specification follows:**

--binding domain. The steroid hormone ecdysone triggers coordinate changes in tissue development that results in metamorphosis, and ecdysone has been shown to bind to EcR. Koelle *et al. Cell* 67: 59-77, 1991. The plant-produced analog of ecdysone, muristerone, also binds to the ligand binding domain of EcR. Other chemicals, such as the non-steroidal ecdysone agonists RH 5849 (Wing, *Science* 241: 467-469 (1988)) and RH 5992 (tebufenozide), the latter known as the insecticide MIMIC<sup>®</sup>, also will act as a chemical ligand for the ligand binding domain of EcR. The EcR and its ligand binding domain have been found in the present invention to be particularly useful for controlling target polypeptide expression in plant cells, as described in the examples below.—

**A marked up version of paragraph 2 on page 16 of the specification follows:**

--Tissue-specific or tissue-preferential promoters useful in the present invention in plants, particularly maize, are those which direct expression in root, pith, leaf or pollen. Such promoters are disclosed in [U.S. Serial No. 07/951,715] Patent No. 5,625,136, herein incorporated by reference in its entirety. Also useful are promoters which confer seed-specific expression, such as those disclosed by Schernthaner *et al.*, *EMBO J.* 7: 1249 (1988); anther-specific promoters

incorporated by reference in its entirety; anther (tapetal) specific promoter B6 (Huffman *et al.*, *J. Cell. Biochem.* 17B: Abstract #D209 (1993)); pistil-specific promoters such as a modified S13 promoter (Dzelkalns *et al.*, *Plant Cell* 5:855 (1993)).—

**A marked up version of paragraph 3 on page 16 of the specification follows:**

--Also useful in the present invention are chemically-induced promoters. Particular promoters in this category useful for directing the expression of the receptor polypeptides or target polypeptide in plants are disclosed, for example, in [U.S. Serial No. 08/181,271] Patent No. 5,614,395, herein incorporated by reference in its entirety.—

**A marked up version of paragraph 1 of page 19 of the specification follows:**

--Serial No. 08/008,374, herein incorporated by reference in its entirety. An additional preferred embodiment is the protoplast transformation method for maize as disclosed in European Patent Application EP 0 292 435, as well as in [U.S. Serial Number 08/024,875] Patent No. 5,350,689, hereby incorporated by reference in its entirety. One particularly preferred set of embodiments for the introduction of the expression cassettes of the present invention into wheat by microprojectile bombardment can be found in U.S. Serial No. 08/147,161, herein incorporated by reference in its entirety.—

**A marked up version of claim 26 follows:**

26. (Amended/Marked up) A receptor expression cassette for use in a method of controlling gene expression in a plant, comprising:
- a) a 5' regulatory region capable of promoting expression in a plant cell;
  - b) an operably linked coding sequence encoding a receptor polypeptide comprising a

ligand binding domain and a DNA binding domain, wherein said receptor polypeptide is a member of the Class II steroid and thyroid hormone superfamily of nuclear receptors; and

- c) a 3' terminating sequence.

**A marked up version of claim 28 follows:**

28. (Amended/Marked up) The receptor expression cassette of claim [27] 26 wherein said receptor polypeptide is Ecdysone Receptor.

**A marked up version of claim 31 follows:**

31. (Amended/Marked up) The receptor expression cassette of claim 28 wherein said DNA binding domain is heterologous with respect to said receptor polypeptide [further comprises a heterologous DNA binding domain].

**A marked up version of claim 32 follows:**

32. (Amended/Marked up) The receptor expression cassette of claim 31 wherein said heterologous DNA binding domain is the DNA binding domain from the GAL4 protein of yeast.

**A marked up version of new claims 37-41 follows:**

37. The receptor expression cassette of claim 26 wherein said receptor polypeptide further comprises a heterologous transactivation domain.
38. The receptor expression cassette of claim 26 wherein said DNA binding domain is heterologous with respect to said receptor polypeptide.



39. A plant transformation vector comprising the receptor expression cassette of claim 26.
40. A plant transformed with the plant transformation vector of claim 39.
41. Seed from the plant of claim 40.

TOP SECRET

**REMARKS**

Continuing data has been inserted on the first page beneath the title.

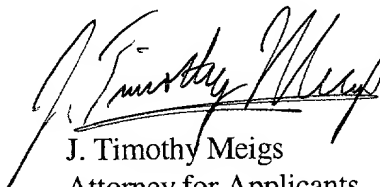
In a parent case, the Examiner noted that the trademark MIMIC should be accompanied by the generic terminology. Accordingly, the specification has been amended to insert the common name for MIMIC, "tebufenozide". Applicants suggest that this does not amount to an addition of new matter, for the compound is referred to in the specification by its trade name MIMIC as well as by development code RH-5992. All of these names, tebufenozide, MIMIC, and RH-5992, are synonymously referred to in Tomlin, E.D.S., ed. The Pesticide Manual, Eleventh Edition, British Crop Protection Council: Surrey, UK, 1997, pp. 1147-1148 (attached hereto as Exhibit A).

The specification has also been amended to update the status of several referenced application serial numbers.

Claims 26, 28, 31, and 32 have been amended. Claims 1-25, 27, and 36 have been cancelled without prejudice or disclaimer. New claims 37-41 have been added. Thus, the pending claims are 26, 28-35, and 37-41.

The Examiner is respectfully requested to enter the above amendments before calculation of the claim fee and commencement of substantive examination. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call Applicants' undersigned attorney.

Respectfully submitted,



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